# SynthImmunol_NMU Notebook No.2

**Time: 2024.09.10-2024.09.15**

1. **Experiment:** Construction of in situ hepatic tumor models
2. **Time:** 2024.09.10-2024.09.15
3. **Member:** Xinyu Zhu, Song Zhang
4. **Material:**

| Name | Supplier or Formulation |
|---|---|
| Animal scales | Shimadzu |
| High-throughput in vivo optical imaging system for small animals | PerkinElmer |
| Vetbond Tissue Adhesive | 3M |
| BALB/c Rag2$^{KO}$ IL2rg$^{KO}$ mice (NCG Mice) | GemPharmatech Co., Ltd |
| PBS | Solabio Biotechnology Co., Ltd. |
| L-glutamine | GIBCO |
| FBS (fetal bovine serum) | GIBCO |
| streptomycin | GIBCO |
| DMEM medium | GIBCO |
| disposable 15 mL and 50 mL centrifuge tubes | Corning |
| cell culture plates and dishes | Corning |
| an Eppendorf 5804R high-speed centrifuge and mini centrifuge | Eppendorf |
| FACS Calibur flow cytometer | BD |

5. **Method:**

(1) Cell Culture: The HCC cell line HepG2, were cultured in DMEM medium supplemented with 10% fetal bovine serum, 100 mg/mL penicillin, and 100 mg/mL streptomycin and maintained in a 37°C incubator with 5% $CO_2$.

(2) Cell Preparation: HepG2 cells were seeded in 10 cm culture dishes using DMEM medium and incubated at 37°C in a 5% $CO_2$ atmosphere until the cells reached the logarithmic growth phase.

(3) Harvesting Cells with Trypsin**:** 1 mL of 0.25% trypsin was added to each 10 cm dish for digestion for one minute. Afterward, 2 mL of DMEM containing 10% fetal bovine serum was added to terminate the digestion. The cell suspension was transferred to a 15 mL centrifuge tube, and 5 mL of PBS was added to wash the dish. The solution was collected into the 15 mL tube, centrifuged at 1000 r/min for 5 minutes, and the supernatant was discarded. The HepG2 cells were then resuspended in PBS, centrifuged again at 1000 r/min for 5 minutes, and the wash was repeated twice.

(4) Cell Counting: The harvested HepG2 cells were resuspended in PBS, counted, and adjusted to a final concentration of $2\times10^7$ cells/mL.

(5) Anesthesia of Mice: Each mouse received an intraperitoneal injection of the anesthetic pentobarbital (150-200 μL), and the injection site was disinfected with 75% ethanol prior to administration.

(6) Injection of the HepG2 Cells: Following anesthesia, a transverse incision of approximately 1 cm was made under the xiphoid process in NCG mice. The abdominal cavity was gently compressed laterally to expose a portion of the liver. Using a 1 mL insulin syringe, 100 μL of the prepared cell suspension was injected intrahepatically into the nude mice. Concurrently with the withdrawal of the needle, an assistant applied a drop of Vetbond Tissue Adhesive to promptly seal the puncture site, preventing hemorrhage and the escape of tumor

cells that could lead to peritoneal seeding. The liver was gently returned to the abdominal cavity using a cotton swab moistened with saline, and the incision was closed. Post-surgery, the mice were placed in a warm environment illuminated by incandescent light until they awakened naturally. Subsequent monitoring of tumor volumes was conducted using small animal imaging.

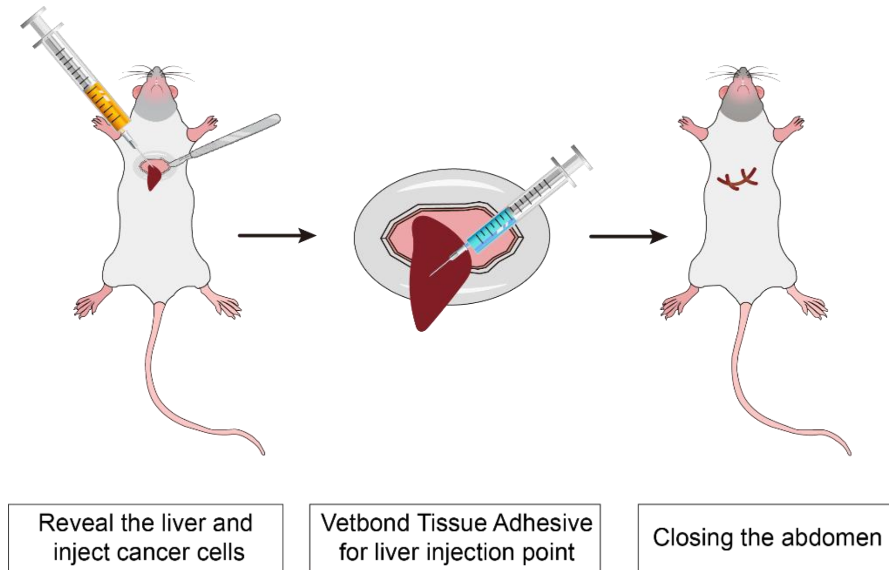


**Fig.1** The in-situ tumor-bearing mouse model construction

To evaluate the impact of inhibitory signal-activated CAR-NK cells on in vivo anti-tumor activity, an in-situ tumor-bearing mouse model of liver cancer was established by inoculating Hep G2 cells into NCG mice.